8162 P

Coagulating Power of Bothrops Atrox Venom on Hemophiliac Blood.

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Barnet and Macfarlane[1] have recently reported on the use of snake venom for local hemostasis. Their best results were obtained with venom of the Russel viper. We have been working along similar lines with the venom of the Bothrops atrox (Fer-de-Lance). This reptile is a native of Central and South America and is fairly easy to obtain and keep in captivity. Thus, unlike the Russel viper venom, a plentiful and relatively cheap supply of this venom is readily available.

Two different samples of the dried venom were obtained through the courtesy of Dr. R. P. Connor, of the United Fruit Co. The samples represented pooled venoms from "milkings" carried out at the Serpentarium, in Tela, Honduras, several years ago. In the experiments, the venom was diluted with physiologic saline.

The minimum lethal dose for pigeons (intravenous route) weighing 250-350 gm. varied from 0.2-0.25 mg.; the minimum lethal dose in rabbits (intravenous route) of approximately 2 kilos, was 0.015-0.02 mg. These figures were determined on a large series of pigeons and rabbits with both samples.

V. Brazil gives 0.01 as the m.l.d. for pigeons and 0.07 mg. as the m.l.d. for rabbits We have consistently found, as can be seen from our figures, that pigeons were much more resistant than rabbits to the toxins (neurotoxins) of the venom.

The haemorrhagine content of the Bothrops atrox venom was titrated according to the method of Witebsky, Peck, and Neter.[2] One sample failed to elicit hemorrhage in a 1% solution, while the other sample produced a moderate amount of echymosis in 3 minutes when 1 cc. of the 1% solution was added to the chicken embryo. It was, however, decidedly poorer in hemorrhage-producing substance than Moccasin venom.

The coagulating power of Bothrops atrox venom was tested on the blood of 2 hemophiliacs: L.E., age 25, whose clotting time was

[1] Macfarlane, R. G., and Barnet, B., *Lancet*, 1934, **2**, 985.

[2] Witebsky, E., Peck, S., and Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 722.

58-60 minutes and V.B., age 36, whose clotting time varied from 1 hour and 40 minutes to 1 hour and 46 minutes. The coagulation time was determined according to the method of Lee and White. To 2 cc. of hemophiliac blood, 0.5 cc. of the Bothrops atrox venom was added in dilutions up to 1:10,000,000, and the time of clot formation was noted.

The optimum concentration of the Fer-de-Lance venom, for coagulation was in the 1:10,000 dilution. Since 0.5 cc. of the venom was added to 2 cc. of blood, the final dilution was 1:50,000. It is of interest to note that the coagulation power of the venom increased with the dilution up to 1:10,000 and then diminished. It took a much longer time to clot the blood of the older hemophiliac whose clotting time was much longer than that of the younger man. The clots when formed, were firm and retracted well. In the case of L. E. it took only 15 seconds to cause the formation of a firm clot on the addition of 0.5 cc. of the 1:10,000 dilution. The venom was still active on this patient's blood in a dilution of 1:1,000,000, 1:5,000,000 decreasing the coagulation time by more than one-half.

When one compares the optimum which we obtained with the report of Barnet and Macfarlane on the Russel viper, it seems that the Fer-de-Lance venom was apparently more efficient in producing a rapid coagulation, since the coagulation time in their patient, as obtained by their method, was only 22 minutes. To test the possibility that Moccasin venom might also act as a coagulant in high dilutions, 0.5 cc. of Moccasin venom in dilutions of 1:2,000, 1:3,000, 1:5,000, 1:10,000, 1:15,000, and 1:20,000 were added to 2 cc. of the blood of L.E. (clotting time 60 minutes). 0.5 cc. of a 1:10,000 dilution of Moccasin venom kept the blood fluid past the 60-minute period, while 0.5 cc. of the 1:20,000 dilution acted like a saline control, in that it either produced no change in the clotting time or prolonged it somewhat.

In order to compare the coagulating action of Bothrops atrox venom with tissue extracts, a rabbit lung was perfused to eliminate as much blood as possible and the lung tissue was dried to a powder in the desiccator. A 1% solution of the lung powder in physiologic saline was made. The blood of V.B., a hemophiliac, used in the previous experiment, whose coagulating time, April 19th, was one hour, was used. The Bothrops atrox solution was 61 days old. As before, 0.5 cc. of a 1:10,000 and a 1:100,000 dilution of the venom was added to 2 cc. of V.B.'s blood, and to a parallel series of 2 cc. quantities of this patient's blood, was added 0.5 cc. of a 1:10,000 and a 1:100,000 lung powder solution. Clotting occurred in 1½

minutes with the 1:10,000 dilution, and in 4 minutes with the 1:100,000 dilution of venom. The 1:10,000 dilution of lung powder caused clotting in 47 minutes, while the 1:100,000 dilution showed no clotting up to the end of the experiment. To test the activity of the lung powder, 0.5 cc. of the original 1% solution of lung powder was added to 2 cc. of the hemophiliac blood, and a firm clot was formed in 6 minutes. The clot following the addition of venom was firm and contracted very rapidly after clotting began, even with the 1:100,000 dilution. The clot formed with the lung powder was soft and easily broken up, and there was no contraction. In spite of the fact that the clotting time of V.B. had decreased, it took 1½ minutes for the 0.5 cc. of the 1:10,000 dilution to produce clotting. This was due to the fact that in the previous experiment a fresh solution of the venom was made, and in 61 days since the preparation of the 1:10,000 venom dilution, there was deterioration of its coagulating properties.

Since the venom is intended for use as a local hemostatic and since venoms often contain bacteria, the effect of filtration through a Zeiss or a Berkefeld filter on coagulating power, was studied. In both instances, filtered solutions were sterile. While the toxicity (neurotoxicity) was reduced as tested on pigeons the coagulation power of the venom was not interfered with as far as could be determined on hemophiliac blood. This confirms similar observation by Barnet and Macfarlane with the Russel viper.

8163 P

Time Curve of Insulin Action in the Depancreatized Dog.

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Scott and Dotti[1] have made an extended study of blood sugar lowering effects of insulin given subcutaneously to rabbits. They have concluded that the results, when expressed as the fraction of sugar removed, are independent of the initial blood sugar while the absolute value of blood sugar after a given time interval or the absolute value of the fall in blood sugar is dependent on the initial value.

[1] Scott, E. L., and Dotti, L. B., *Arch. Int. Med.*, 1932, **50**, 511.